REMARKS

Initially, Applicant would like to once again thank Examiner Bausch for her time in conducting a telephone interview on March 29, 2006. Additionally, Applicant would like to thank the Examiner for welcoming comments after the Office Action of July 26, 2006 and offering to review them in a telephone conversation on December 29, 2006.

1. Objection to claim 56 Compliance with 37 CFR 1.121

The Examiner noted that claim 56 did not comply with 37 CFR 1.121. The claim has been amended accordingly. More specifically, the deleted phrase, "without removal of unreacted probes," has now been appropriately denoted rendering the rejection moot.

2. Rejection of Claims 56-75 under 35 U.S.C. 112, first paragraph.

Claims 56-75 were rejected under 35 U.S.C. 112, first paragraph for the reasons of record. Specifically, the Examiner argued that the recitation of Claim 56 "wherein a positive signal is generated only when two or more components are co-localized, thus allowing detection" is not supported by the Specification.

Applicant respectfully directs the Examiner to paragraph [0046] of the Specification wherein it states, "[i]n the methods described herein, detection is based on the use any one of the components detectably labeled: the probe DNA, the RecA, the MutS, (or SSB, discussed below). The label may be any suitable detectable label, e.g., a fluorophore, a chromophore, a radionuclide, biotin, digoxigenin, etc."

Applicant directs the Examiner to paragraph [0103] of the published application wherein the Specification states, "[i]n this method detection of mutations and SNPs is accomplished by detecting the co-localization of either (a) RecA and MutS, (b) probe DNA and MutS or (c) RecA, MutS and probe DNA." ***(emphasis added)***

Applicant also directs the Examiner to paragraph [0113] wherein the Specification states, "[t]he flow cytometer is set to detect as a signal the ***simultaneous***

presence of both labels (that on the MutS and that on the RecA and/or probe) or the presence of a "third" color created by the juxtaposition of the two (or three) labels. The presence of such signals is an indication of the presence in the sample of sequences differing from the probe by one or a few single mismatches or unpaired bases."

Applicant further directs the Examiner to paragraph [0114] wherein the Specification states, "[t]he power of the RecA/MutS method described herein is that the background signals are very low, and RecA+MutS (or MutS+DNA probe or MutS+RecA+DNA probe) will be found together only under conditions in which RecA-coated oligonucleotide probe has bound to test DNA in a way that creates a heteroduplex with a mismatched or unpaired base."

Applicant also directs the Examiner to paragraph [0118] wherein the Specification states, "[i]n another generally applicable embodiment of this invention, MutS may be immobilized, and either the probe of the RecA may be detectably labeled. In this embodiment, binding of the probe or RecA to immobilized MutS is indicative of one or more mismatches or unpaired bases in the D-loop structure formed between the probe and test DNA."

Applicant further directs the Examiner to paragraph [0069] wherein, Figures 1-7 are described and the Specification states, "FIGS. 1-7 are schematic representations of the RecA+MutS mutation/SNP detection method including various detection modalities." In particular, paragraph [0070] of Figure 1 depicts the general concept of the invention wherein it states that, "an oligonucleotide "probe" to which is added in Step (1) the RecA (smallcircle) protein. RecA coats the probe to form a "RecA filament." In Step (2) RecA filament is added to test DNA and allowed to form a triple stranded or "D-loop" structure. In Step (3), the MutS protein is added. If the probe is identical to the test DNA sequence, a perfectly paired duplex ("no mismatch") is formed and the MutS does not bind (left). If there are one or more sequence differences between the probe and test DNA sequences, a heteroduplex is formed containing one or more mismatches or unpaired bases ("Mismatch (SNP)") and MutS binds to that heteroduplex."

Throughout the Specification, specific detection methods in a variety of different embodiments are described in detail. For instance, flow cytometry is described in paragraphs [0113] to [0116], standard genotyping is described in paragraph [0123], a variety of other methods utilizing the formation of heteroduplex DNA [0127], and the like. Unreacted components in the reaction mix are not scored and independent claim 56 includes the claim limitation, "wherein a positive signal is generated only when two or more components are co-localized, thus allowing detection."

As such, the Specification clearly teaches that one of the components must be labeled (herein probe DNA, RecA, MutS or SSB) and that subsequent detection is dependent on the "co-localization" of two or more components (herein RecA and MutS, probe DNA and MutS or RecA, MutS and probe DNA) and the Specification teaches the detection of those two or more components. ***(emphasis added)*** In light of the foregoing, one skilled in the art would realize that a positive signal is generated when there is co-localization of two or more components of the present invention with one such component being labeled. More specifically, the co-localization of RecA and/or probe DNA with MutS is the very heart of the present invention and the labeling of one or more of these three components allows for the detection of such co-localized components.

Accordingly, Applicant argues that the specification describes the claimed invention in such a way as to convey to one of skill in the art that the Applicant had possession of the claimed invention consistent with 35 U.S.C. 112, first paragraph and relevant case law. (Lockwood v. American Airlines, Inc. 107 F. 3d 1565, 41 USPQ2d 1061 (Fed Cir. 1997); and In re Gostelli, 872 F.2d 1008, 10 USPQ2d 1614 (Fed. Cir. 1989)). As such, Applicant respectfully requests withdrawal of the Examiner's rejection of Claims 56-75 under 35 U.S.C. 112, first paragraph.

3. Rejection of Claims 56-75 under 35 U.S.C. 103(a)

Claims 56-75 were rejected under 35 U.S.C. 103(a) as being unpatentable over Kigawa et al. ("Kigawa") in view of Nolan et al. ("Nolan") for the reasons of record.

As pointed out by the Examiner, Kigawa does not teach the use of MutS protein with RecA for the detection of chromosomal aberrations, but the Examiner argues that one of skill in the art would have been motivated to improve the method of detecting the double stranded target nucleic acid sequence using the probe/RecA hybridization system by Kigawa with the mismatch binding protein, MutS, immobilized to microspheres taught by Nolan.

As discussed extensively in the telephone interview conducted March 29, 2006 and a variety of previously filed responses to Office Actions, Applicant respectfully traverses the rejection.

Briefly, Applicant characterizes Kigawa as teaching the use of RecA for determining the presence of large scale chromosomal type mutations, insertions deletions, and the like. Such insertions and deletions detectable by the Kigawa method are not the MutS-recognizable SNPs, insertions or deletions of the present claims. Applicant is highly skeptical that the methods of Kigawa would be capable of distinguishing such small variations from the wild-type sequences.

Kigawa also teaches the use of probes, "with homology of at least 90% to 95% with the target nucleic acid sequence." A person of ordinary skill in the art would appreciate that such homology levels would not allow detection of the SNPs, insertions and/or deletions of the present invention. Kigawa teaches away from the present invention wherein the probe is designed to avoid successful homology searching, as in Kigawa, unless there is nearly perfect homology, such as in a SNP, insertion and/or deletion. Kigawa also fails to suggest the use of a probe with an intentional mismatch and, in contrast, actually directs the use of probes that are identical to the target sequence that they are attempting to detect. Kigawa also does not provide any motivation for a person of ordinary skill in the art to consider the use of MutS.

Applicant characterizes Nolan as teaching the use of MutS for determination of small errors in single-stranded DNA. Nolan does not suggest that the three-stranded D-loop structure formed by RecA homology searching can be a substrate for MutS binding or to combine the MutS methods with any other method, such as RecA. Further Nolan did not suggest the labeling of the MutS, as in the present invention. Nolan only fluorescently labeled the target DNA.

The Examiner contends that one of ordinary skill in the art would have had a reasonable expectation of success because it was well known that MutS binds triple helix structures. As support, she recites from Patent No: 6,120,992 wherein it states, "[t]he present invention includes the use of an immobilized MBP to bind to and detect any of the following duplexes or triplexes containing the base pair mismatch or other mispairing which the MBP is capable of recognizing: a DNA-PNA duplex, a PNA-PNA, a RNA-PNA duplex, DNA triplex containing a third strand of either PNA or RNA, a DNA-RNA-PNA triplex, a PNA-PNA-DNA triplex." In contrast, the present invention is directed to triplex helix DNA structures only. ***(emphasis added)*** Rather than supporting a reasonable expectation of success, as the Examiner contends, the recited reference in fact teaches away from MutS binding triplex DNA structures as it specifically teaches that triplex helix structures require one non-DNA strand for binding to occur. (herein PNA) Therefore, the reference provides no suggestion to bind MutS to a DNA triplex structures.

Applicant would also like to clarify two points in the July 26, 2006 Office Action, First, the Examiner states, "[t]he instant specification teaches the mismatch repair system of E. coli recognizes mismatches in the hybrid overlaps, which states that prior to the invention it was known that the mismatch system binds hybrid structures (which includes two, three and four stranded structures)(see page 2, lines 23-30). Applicant correctly references this paragraph in the published paragraph below in its entirety:

[0008] MutS and RecA are bacterial proteins involved in DNA repair and genetic recombination and have been best characterized in E. coli. MutS is

the mismatch recognition and binding protein of the E. coli mismatch repair system, which functions to repair errors made by DNA polymerase during DNA replication. The system also recognizes mismatches in the ***hybrid overlaps*** *created in the initial steps of genetic recombination and acts on such mismatch containing regions to abort recombination. Thus, the mismatch repair system is an editor both in DNA replication and genetic recombination and assures high fidelity in both processes. (The editing of recombination is essential to avoid chromosomal rearrangements, to allow successful meiosis and to erect a genetic barrier between closely related species.)*

As can be seen from the full recital of this paragraph directly from the published application, nowhere does Applicant state that hybrid structures include three and four stranded structures as stated by the Examiner. Applicant strenuously objects to the Examiner's conclusory remark as it feels hybrid overlaps are **duplex DNA** composed of one strand from each of two recombining parents and, as such, are never thought of as including three or four stranded structures. Applicant directs Examiner's attention to Exhibit 1, wherein the classical reference states, "[t]hese hybrid or hetereoduplex regions are made up of one strand from each parental molecule...." Hence, they are two strands of DNA only and not three or four strands. Applicant can furnish additional references further providing support for the definition of hybrid structures according to one skilled in the art if the Examiner wishes; the undersigned is happy to e. mail pdf versions to the Examiner upon request.

Secondly, Applicant objects to the Examiner's characterization that "one of ordinary skill in the art would have been motivated to use MutS with RecA for detection of mismatches because both MutS and RecA are part of the mismatch repair system of E. coli." Applicant vehemently disagrees with the statement that both MutS and RecA are part of the mismatch repair system of E. coli. As this mischaracterization appears to provide the Examiner support for her contention that there exists a motivation to combine the teachings of Kigawa and Nolan, Applicant would like to briefly explain its argument.

Briefly, Applicant directs the Examiner's attention to paragraph [0010] of the Background of the published application that describes the naturally-occurring action of RecA and MutS. Herein, Applicant states that, "RecA, a bacterial recombinase which has been best characterized in E. coli, is the key player in the process of genetic recombination, in particular in the search and recognition of sequence homology, and the initial strand exchange process." Further, the Background states, "Recombination is initiated when multiple RecA molecules coat a stretch of single stranded DNA (ssDNA) to form what is known as a RecA "filament." This filament, in the presence of ATP, searches for homologous sequences in double stranded DNA (dsDNA). When homology is located, a three stranded (D-loop) structure is formed wherein the RecA filament DNA is paired with the complementary strand of the duplex." Applicant then directs the Examiner's attention to the role of MutS, wherein the Background states in this same paragraph, "If pairing is not perfect, i.e., if there are mismatches or unpaired bases in the newly created duplex, MutS can bind to these structures and mobilize the other proteins of the mismatch repair system which act to abort the recombination event by removing the filament DNA and restoring the original duplex."

Applicant objects to Examiner's conclusory remarks pertaining to MutS and RecA as both being part of the mismatch repair system of E. coli and, in contrast, argues that MutS has no interaction with RecA during the repair or replication errors. The function of MutS is to repair such errors and that is arguably its primary function to do so. In contrast, RecA is a recombination protein. Additionally, Applicant reminds Examiner that the remarks above pertaining to the naturally-occurring action of RecA and MutS are very different to the action of both agents in the present invention that benefits from the non-naturally occurring three- and four-stranded D-loops. The Examiner has not pointed to any art that teaches or suggests such systems would work with such unnatural structures.

Based on the foregoing, Applicants argue that the primary reference cannot be considered an adequate legal basis for a *prima facie* obviousness rejection for the reasons discussed above. Kigawa actually teaches away from the present invention and

does not provide any suggestion to look towards Nolan to fill the gaps with respect to the present claims. Nolan teaches the use of immobilized MutS to detect mismatches in duplex DNA but nowhere suggests the detection of mismatches in three- or four-stranded structures. As argued above, one of ordinary skill in the art would not have known that MutS was capable of binding such structures at the time of the filing of this application. Nolan also never suggests detecting MutS, alone or in combination, as only the DNA probes in the Nolan method were labeled. The methods of the present invention are actually unexpected in light of the teachings of Kigawa and Nolan.

As such, Applicant respectfully argue that the combination of Kigawa and Nolan do not produce the claimed present invention and that there is no suggestion or motivation to combine the Kigawa and Nolan references in such a way as to achieve the claimed invention. As such, the Applicant respectfully contends the 103(a) rejection has been improperly made. (In re Vaeck, 947 F.2d 488, 20 USPQ2d 1438 (Fed. Cir. 1991). Applicant further respectfully argues that the Examiner's asserted *prima facie* case is also improper as one of ordinary skill in the art at the time the invention was made would not have reasonably expected the invention to work. (In re O'Farrell, 853 F.2d 894, 7 USPQ2d 1673, 1681 (Fed. Cir. 1988); In re Dow Chem., 837 F.2d 469, 473, 5 USPQ2d 1529, 1531 (Fed. Cir. 1988).

In view of the foregoing arguments and amendments, Applicant respectfully contends that all claims are in a condition for allowance. In the event the Examiner has any questions regarding the Applicant's position, a telephone call to the undersigned representative is requested.

Respectfully Submitted,

December 26, 2006
Date

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APPENDIX 1

PNAS
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A Mechanism for Initiation of Genetic Recombination

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Genetics

A mechanism for initiation of genetic recombination

(palindrome/crossed strand connection)

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Communicated by Matthew Meselson, June 9, 1975

ABSTRACT A mechanism for the initiation of genetic recombination is proposed. Its key features are the pairing, nicking, and cross-annealing of palindromic loops, i.e., structures formed by DNA with sequences of inverted complementary repeats. This mechanism may provide a simple, yet specific means of producing crossed strand connections between homologous DNA duplexes to form structures which can be intermediates in the process of genetic recombination.

Genetic and physical studies indicate that genetic recombination in both prokaryotes and eukaryotes involves the breakage and rejoining of homologous DNA molecules, which is initiated with, or at least accompanied by, the formation of regions of hybrid DNA (for review see refs. 1–3). These hybrid or heteroduplex regions are made up of one strand from each parental molecule and are the presumed sites of gene conversion, i.e., recombination by mismatch repair (4). Analysis of unselected tetrads in yeast (5) suggests that all meiotic recombination is associated with the formation of hybrid DNA.

Several models of genetic recombination have been proposed which involve the formation of hybrid DNA in their initial steps (see ref. 2). A crossed strand exchange between homologous DNA molecules (Fig. 1D) has been proposed by Holliday (4) as one possible intermediate in the formation of hybrid DNA. Model building by Sigal and Alberts (6) has shown that such a crossed strand connection can be formed between DNA duplexes without disruption of either base pairing or stacking and can migrate along the duplexes, perhaps by rotary diffusion (7), to produce extensive regions of hybrid DNA in both molecules. Strand equivalence in the connected structure allows the duplexes to undergo isomerization, i.e., the interchanging of crossing and noncrossing strands (Fig. 1D and D′), which can result in the formation of nearly equal numbers of crossover and noncrossover molecules (6, 8, 9).

Since crossed strand connections are a part of several models of genetic recombination and appear to be feasible from a physical standpoint, the manner in which they arise and the genetic consequences of their formation may be of considerable importance to the understanding of genetic recombination. We shall describe here a possible mechanism for the formation of crossed strand connections at specific sites on homologous DNA duplexes.

THE MECHANISM

This mechanism postulates that recombination initiates at and depends upon palindromic (i.e., inverted complementary repeat) sequences in the DNA (Fig. 1A) capable of forming the characteristic structures (palindromic loops) shown in Fig. 1B. Model building (10) shows that such structures must have at least two unpaired bases at the apex of the loop which could form base pairs with the complementary bases in an identical loop. This pairing, as well as formation of the palindromic loops themselves, may be facilitated or stabilized by a recombination protein. If nicks are introduced, possibly by the same protein, at sites identical with regard to structure, sequence, and polarity, cross-annealing will form a double-stranded bridge between the two molecules (Fig. 1C). Cross-annealing depends on the denaturation and renaturation of a short region of the palindromic loops. At present we are unable to imagine any means other than random denaturation and renaturation by which this exchange could be promoted as a consequence of the properties of the nucleic acid structure itself, but we recognize that such an exchange could be facilitated by proteins capable of lowering the energy barrier for the denaturation step (see ref. 11). It is important in this regard to note that the stability of the double-stranded bridge is greater than that of the individual palindromic loops due to its greater length of double helix and complete base pairing and stacking, so that once formed, it should not easily revert to individual loops. Once cross-annealing has formed the double-stranded bridge, limited rotation of the two stem DNA molecules about the axis of this bridge to unwind the annealed palindromic loops, accompanied by rotation of the stem duplexes about their helical axes to wind the outside loops and double-stranded bridge back into the stem, will produce a cross connected structure with no unpaired bases and a short region of hybrid DNA in each of the two molecules (Fig. 1D). This sugar-phosphate bridge between the two molecules can then migrate by rotary diffusion (7) and thus produce a variable length of hybrid DNA in both molecules. In addition, it is possible for the structure to undergo isomerization (refs. 6, 8, and 9; Fig. 1D′) to produce nearly equal numbers of crossover and noncrossover molecules following resolution. Resolution depends on the occurrence of additional nicks or breakage of the cross connection (see *Discussion*).

Because the recognition structure in this mechanism has a palindromic sequence, pairing and formation of the cross connected structure would be expected to occur with equal frequency between identical and complementary strands of the homologous DNA duplexes. A cross connected structure formed by the pairing of complementary strands would normally be resolved by falling apart at the nicks, since migration of the cross connection would be limited to the short region of the palindromic sequence by the lack of any additional homology. To insure that such resolution occurs and that improper recombinants are not formed, it is essential

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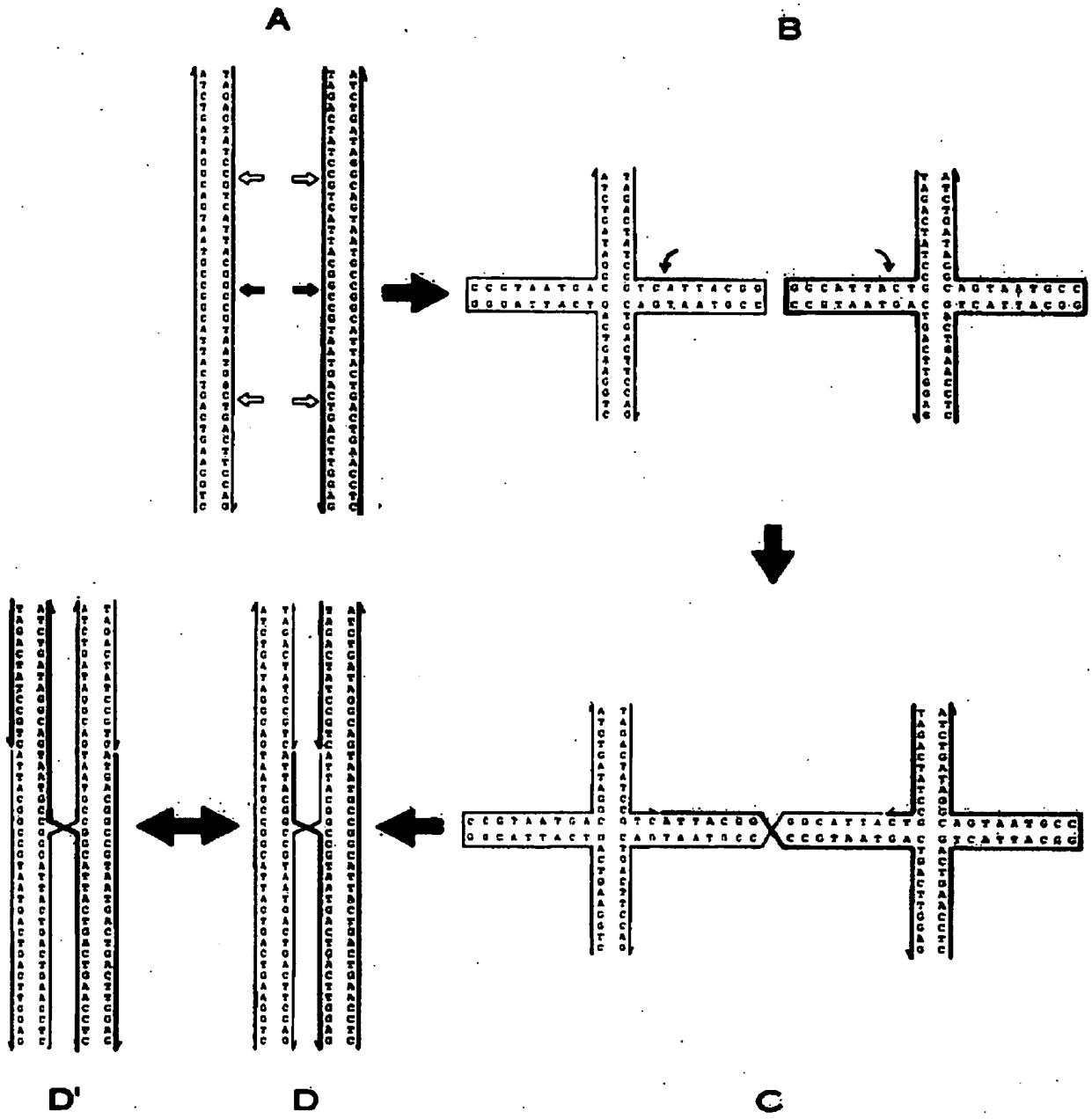

FIG. 1. (A) Homologous DNA duplexes with palindromic sequences. Solid arrows: apex of palindromic loop. Open arrows: base of palindromic loop. (B) Duplexes with palindromic loops. At least two bases at each apex are not paired and could facilitate pairing of the palindromic loops by pairing with the complementary bases at the apex of an identical loop. For clarity, this interpalindromic base pairing is not shown in the figure. Arrows indicate possible sites for specific endonuclease action. (C) Cross-annealed palindromic loops. Double-stranded bridge is formed by the introduction of nicks at identical sites on the palindromic loops as indicated in (B) and exchange of strands by denaturation and renaturation. All bases are paired. (D) Cross connected structure. Formed by limited rotation of the stem DNA molecules about the axis of the double-stranded bridge to unwind the annealed palindromic loops, accompanied by rotation of the stem duplexes about their helical axes to wind the outside loops and double-stranded bridge back into the stem. Cross strand connection may migrate by rotary diffusion to form extensive regions of hybrid DNA. (D') Isomerization. Strand equivalence in the cross connected structure allows crossing strands to become noncrossing strands (and vice versa) and consequently form crossover and noncrossover molecules with nearly equal frequency.

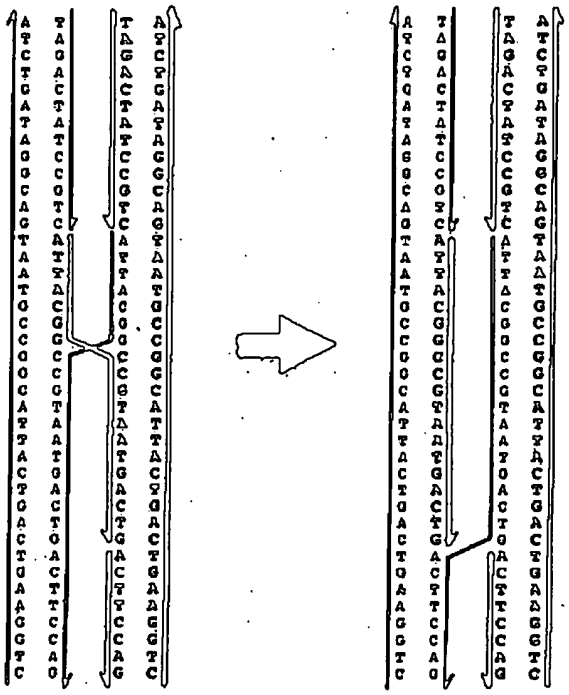

FIG. 2. Formation of a unitary strand connection. Migration of cross connection (left) by rotary diffusion produces a unitary strand connection (right) if a nick in only one strand is encountered.

that the nicks remain unsealed throughout the initial stages of the recombination event.

DISCUSSION

The mechanism for the initiation of genetic recombination proposed here provides for the formation of cross connections between homologous DNA duplexes in a simple, yet specific manner. The recognition sequence can be short and can be the same for all recombination sites within a genome, thus requiring only one sequence specific endonuclease for all nicking associated with the initiation of recombination. Although cross connections may be formed between improper sites, the lack of homology outside the recognition sequence will sharply limit migration of the cross connection and result in the molecules falling apart, as was described for the case of a cross connection formed between complementary strands.

A mechanism for genetic recombination proposed by Sobell (12) also makes use of looped structures for initiating synapsis. However, several features of this mechanism clearly distinguish it from the mechanism proposed here. The loops in Sobell's model are Glaser loops (13) with extensive nonpalindromic sequences at the apex, and his mechanism involves pairing of complementary strands, nicking at nonidentical sequences, and the generation and subsequent annealing of single-stranded ends leading to the formation of a structure with two cross connections.

A prediction of our mechanism is that recombination initiates at specific sites and consequently exhibits polarity. Polarity in the frequency of gene conversion has been observed in yeast (14) and other fungi (15–18). Palindromes, i.e., segments of single-stranded DNA resistant to single-strand deoxyribonucleases, have been observed in eukaryotic chromosomal DNA (19) and are found to average several hundred nucleotide pairs in length. Smaller palindromes, which may be preferable as initiation sites for genetic recombination, have been reported to occur in some prokaryotes (see ref. 19) and sequencing data indicate that in the case of restriction enzymes, small palindromic sequences can function as recognition sites for specific endonucleases (20–23).

Since the cross connection formed by the mechanism proposed here originates several base pairs away from the nicks (Fig. 1D) and since migration of the cross connection back to the nicks will result in the duplexes falling apart, hybrid DNA may be formed more often on the same side of the nicks as the original cross connection. Therefore, nicking at sequence specific sites may result in the formation of predominantly one hybrid overlap polarity for all recombination initiating at a given site. Such an asymmetry in the formation of hybrid overlaps has been reported for bacteriophage λ recombination by White and Fox (24).

The cross connected structure initially formed by this mechanism would produce hybrid DNA in both participating molecules and could be resolved by breaking both strands at the cross connection or by introducing nicks at identical sites in front of the migrating connection. If, however, a nick is encountered in only one crossing strand, a structure with a unitary strand connection results (Fig. 2) and migration by rotary diffusion may no longer be possible. Three outcomes of such an event can be envisioned: (*i*). The structure is resolved by breaking the unitary strand connection; (*ii*) isomerization occurs, generating a cross connection where rotary diffusion is again possible; and (*iii*) the unitary strand connection is driven by the concerted action of an exonuclease and polymerase (6, 8) and thus forms hybrid DNA in only one molecule. In those organisms where hybrid DNA usually forms in only one chromatid (25, 26), such a driven unitary strand connection may be the predominant means of producing hybrid DNA. Meselson and Radding (8) have proposed a model for genetic recombination which, unlike the mechanism proposed here, initiates with the formation of a driven unitary strand connection and in a later step involves isomerization to produce a crossed strand connection.

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